

## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1. Transcriptional response of control and Cpt2<sup>A/-</sup> iWAT to adrenergic stimulation at thermoneutrality, related to Figure 1.**

- (A) Figure Legend.
- (B) mRNA expression in iWAT of thermogenic and brown fat enriched genes (n=5).
- (C) mRNA expression in iWAT of *Ppara* and *Ppara* target genes (n=5).
- (D) mRNA expression in iWAT of metabolic genes (n=5).

Data are expressed as mean ± SEM. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001.

**Figure S2. Transcriptional response of control and Cpt2<sup>A/-</sup> iWAT to a high fat diet at thermoneutrality, related to Figure 4.**

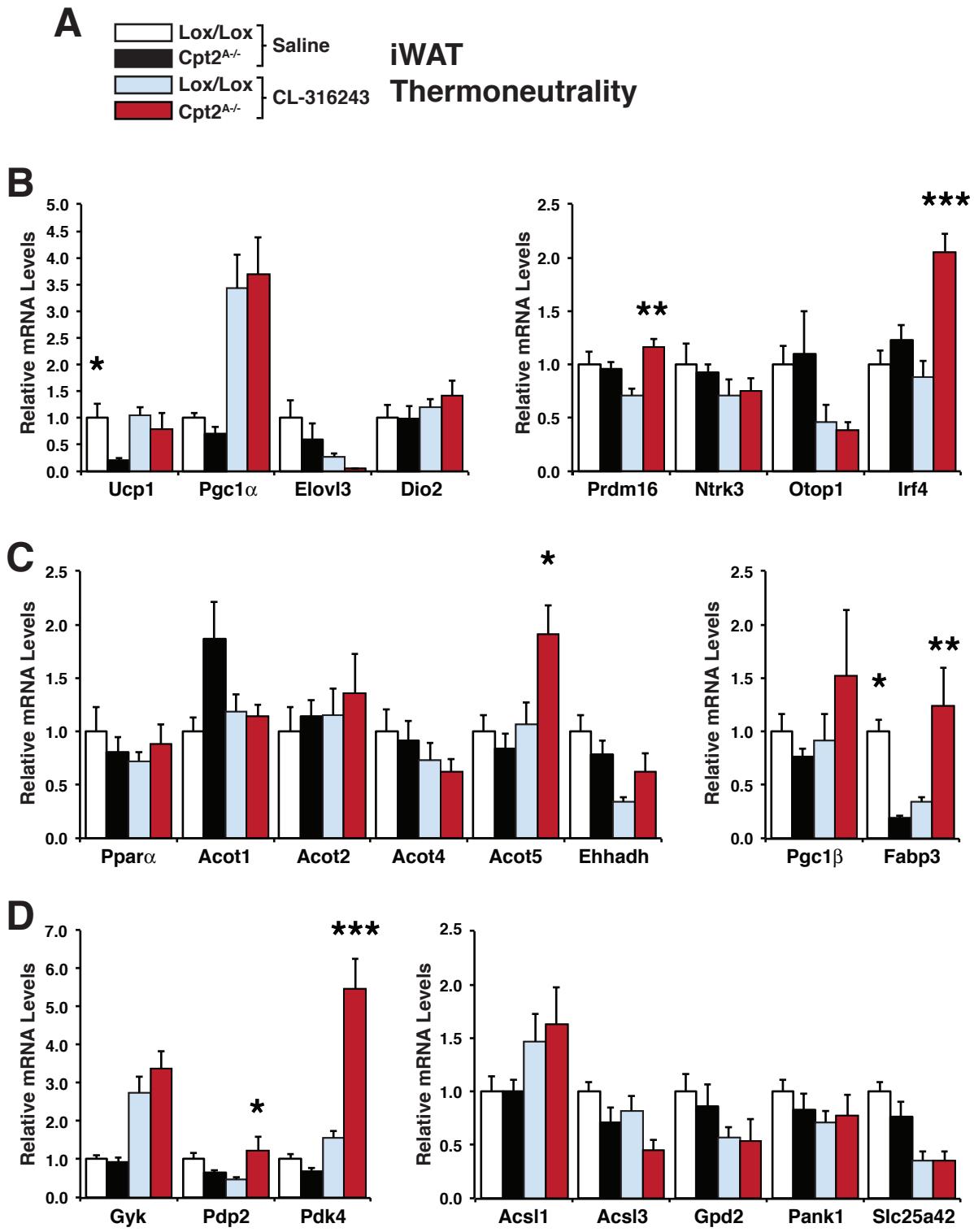
- (A) mRNA expression in iWAT of thermogenic genes (n=5).
- (B) mRNA expression in iWAT of *Ppara* and *Ppara* target genes (n=5).
- (C) mRNA expression in iWAT of interferon responsive genes (n=5).

Data are expressed as mean ± SEM. \*p<0.05.

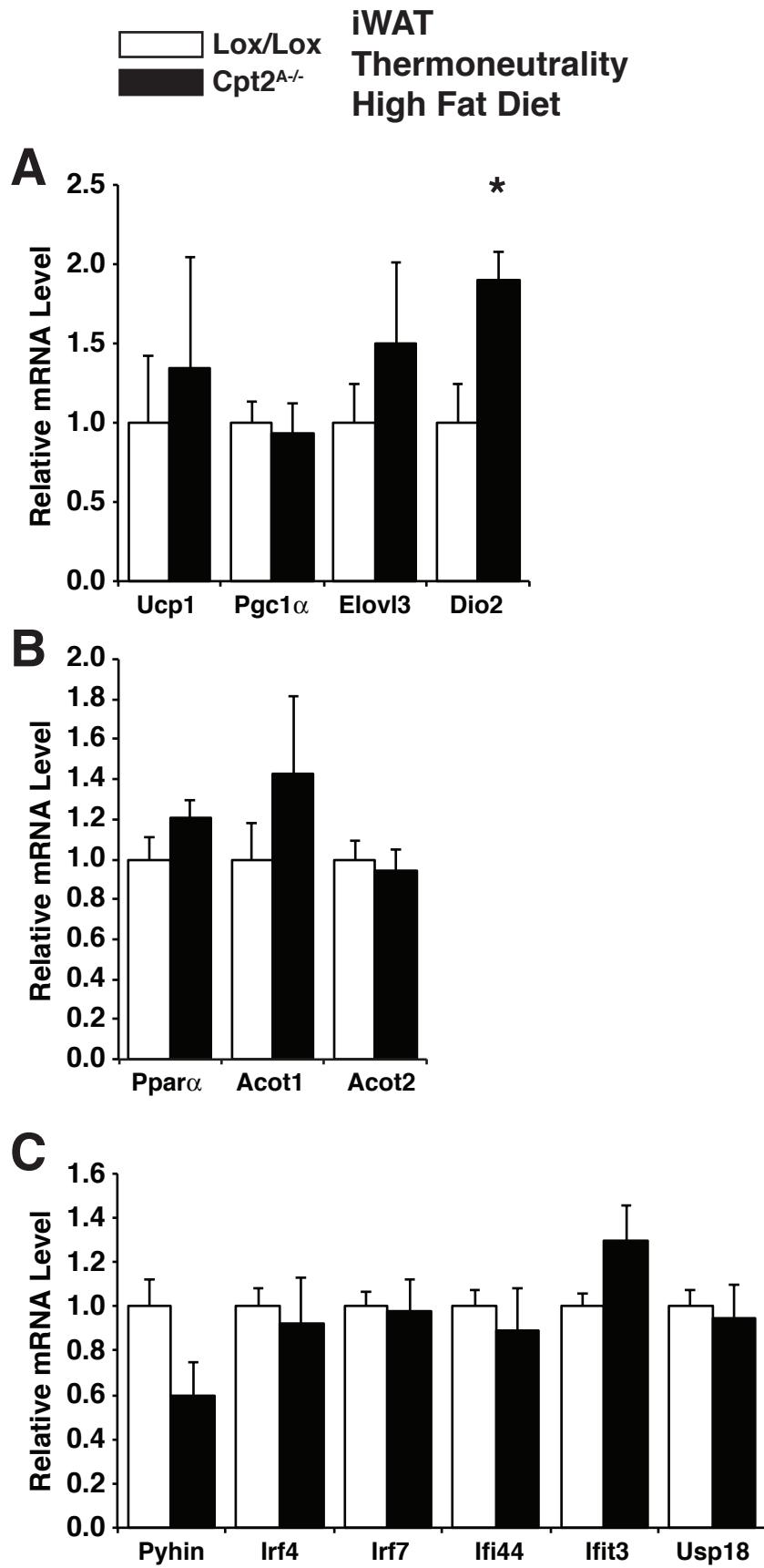
**Table S1. Blood acylcarnitine profile of thermoneutral acclimatized Cpt2lox/lox and Cpt2<sup>A/-</sup> mice before and 1, 2, and 3 hours following CL-316243 (10mg/kg), related to Figure 2.**

**Table S2. Gene abbreviations and primer sequences, related to Experimental Procedures.**

# Figure S1



# Figure S2



**Table S1.** Blood acylcarnitine profile of thermoneutral acclimatized Cpt2lox/lox and Cpt2A-/ mice before and 1, 2, and 3 hours following CL-316243 (10mg/kg), related to Figure 2.

Acylcarnitines (nmol/ml)	Lox/Lox				Cpt2A-/-			
	0	1	2	3	0	1	2	3
Total	24.654 ± 2.462	27.036 ± 0.484	29.56 ± 1.641	27.512 ± 1.377	23.434 ± 2.647	25.364 ± 0.377	27.4 ± 2.325	28.828 ± 1.153
C0	35.074 ± 2.775	21.498 ± 1.35****	24.198 ± 1.216***	22.11 ± 1.741***	28.636 ± 1.592 #	19.814 ± 1.101**	17.604 ± 1.078 #, ***	21.526 ± 1.767*
C2	19.124 ± 1.97	20.51 ± 0.507	22.762 ± 1.462	20.798 ± 1.246	18.042 ± 2.386	19.328 ± 0.177	20.888 ± 2.092	21.884 ± 0.924
C3	0.958 ± 0.124	0.624 ± 0.085*	0.586 ± 0.091**	0.494 ± 0.041***	0.744 ± 0.085	0.408 ± 0.05*	0.438 ± 0.044*	0.44 ± 0.048*
C3-DC	0.174 ± 0.015	0.154 ± 0.013	0.206 ± 0.031	0.144 ± 0.012	0.132 ± 0.023	0.144 ± 0.032	0.152 ± 0.012	0.124 ± 0.022
C4	0.726 ± 0.051	0.53 ± 0.055	0.482 ± 0.07	0.476 ± 0.031*	0.58 ± 0.084	0.568 ± 0.04	0.49 ± 0.069	0.542 ± 0.069
C4-OH	0.154 ± 0.016	0.248 ± 0.033*	0.258 ± 0.012*	0.328 ± 0.031****	0.146 ± 0.026	0.248 ± 0.032*	0.266 ± 0.016**	0.302 ± 0.047***
C4-DC	0.13 ± 0.011	0.126 ± 0.011	0.13 ± 0.022	0.124 ± 0.017	0.156 ± 0.013	0.134 ± 0.01	0.116 ± 0.012	0.128 ± 0.015
C5:1	0.014 ± 0.004	0.024 ± 0.005	0.026 ± 0.006	0.022 ± 0.004	0.018 ± 0.006	0.01 ± 0.003	0.018 ± 0.004	0.014 ± 0.002
C5	0.144 ± 0.019	0.078 ± 0.009***	0.098 ± 0.004*	0.064 ± 0.015****	0.116 ± 0.015	0.094 ± 0.007	0.074 ± 0.012*	0.068 ± 0.012*
C5-OH	0.086 ± 0.011	0.08 ± 0.013	0.072 ± 0.009	0.082 ± 0.02	0.09 ± 0.008	0.07 ± 0.005	0.08 ± 0.021	0.076 ± 0.01
C5-DC/C10-OH	0.02 ± 0.003	0.026 ± 0.004	0.034 ± 0.007	0.03 ± 0.009	0.03 ± 0	0.032 ± 0.007	0.034 ± 0.005	0.028 ± 0.005
C6	0.152 ± 0.012	0.108 ± 0.014	0.118 ± 0.01	0.122 ± 0.02	0.11 ± 0.01	0.136 ± 0.009	0.138 ± 0.009	0.138 ± 0.018
C8:1	0.054 ± 0.007	0.054 ± 0.007	0.068 ± 0.006	0.06 ± 0.008	0.052 ± 0.004	0.048 ± 0.009	0.054 ± 0.005	0.054 ± 0.008
C8	0.058 ± 0.011	0.062 ± 0.006	0.078 ± 0.006	0.07 ± 0.014	0.046 ± 0.01	0.056 ± 0.005	0.06 ± 0.008	0.076 ± 0.005
C10:1	0.062 ± 0.004	0.084 ± 0.01	0.104 ± 0.014*	0.09 ± 0.008	0.072 ± 0.01	0.082 ± 0.008	0.088 ± 0.007	0.118 ± 0.01*
C10	0.03 ± 0.005	0.05 ± 0.004	0.07 ± 0.012***	0.046 ± 0.005	0.05 ± 0.006	0.05 ± 0.003	0.046 ± 0.008	0.066 ± 0.007
C12:1	0.034 ± 0.006	0.072 ± 0.011*	0.058 ± 0.011	0.06 ± 0.004	0.042 ± 0.01	0.058 ± 0.007	0.064 ± 0.002	0.06 ± 0.013
C12	0.062 ± 0.008	0.112 ± 0.007**	0.116 ± 0.014**	0.108 ± 0.01*	0.06 ± 0.008	0.072 ± 0.012	0.086 ± 0.01	0.106 ± 0.012*
C12:1-OH	0.056 ± 0.009	0.056 ± 0.013	0.042 ± 0.006	0.046 ± 0.005	0.07 ± 0.007	0.044 ± 0.008	0.044 ± 0.008	0.058 ± 0.007
C12-OH	0.02 ± 0.004	0.028 ± 0.004	0.028 ± 0.006	0.03 ± 0.004	0.016 ± 0.002	0.018 ± 0.002	0.034 ± 0.005	0.03 ± 0.003
C14:2	0.028 ± 0.008	0.046 ± 0.007	0.072 ± 0.014**	0.054 ± 0.002	0.03 ± 0.003	0.058 ± 0.007	0.052 ± 0.006	0.056 ± 0.015
C14:1	0.072 ± 0.009	0.18 ± 0.028**	0.21 ± 0.019****	0.138 ± 0.017	0.11 ± 0.016	0.126 ± 0.012	0.172 ± 0.027	0.192 ± 0.022*
C14	0.15 ± 0.025	0.364 ± 0.041***	0.428 ± 0.044****	0.392 ± 0.058***	0.192 ± 0.03	0.316 ± 0.012	0.41 ± 0.043***	0.316 ± 0.039
C14:1-OH	0.034 ± 0.009	0.042 ± 0.007	0.056 ± 0.009	0.062 ± 0.014	0.03 ± 0.004	0.044 ± 0.004	0.054 ± 0.007	0.056 ± 0.018
C14-OH	0.034 ± 0.005	0.046 ± 0.008	0.056 ± 0.019	0.052 ± 0.004	0.042 ± 0.007	0.038 ± 0.006	0.04 ± 0.007	0.038 ± 0.006
C16:1	0.142 ± 0.024	0.308 ± 0.031***	0.284 ± 0.007**	0.258 ± 0.027**	0.146 ± 0.017	0.286 ± 0.033**	0.346 ± 0.065****	0.286 ± 0.046**
C16	0.974 ± 0.132	1.442 ± 0.137**	1.442 ± 0.091**	1.548 ± 0.067***	1.13 ± 0.109	1.354 ± 0.046	1.44 ± 0.098	1.67 ± 0.137***
C16:1-OH	0.056 ± 0.014	0.06 ± 0.01	0.078 ± 0.004	0.066 ± 0.01	0.048 ± 0.005	0.066 ± 0.009	0.066 ± 0.008	0.078 ± 0.01
C16-OH	0.042 ± 0.011	0.074 ± 0.013	0.082 ± 0.014	0.082 ± 0.012	0.046 ± 0.007	0.062 ± 0.006	0.074 ± 0.01	0.096 ± 0.016*
C18:2	0.22 ± 0.031	0.34 ± 0.033	0.342 ± 0.041	0.35 ± 0.02	0.268 ± 0.043	0.338 ± 0.022	0.4 ± 0.045	0.408 ± 0.048*
C18:1	0.478 ± 0.052	0.678 ± 0.043*	0.728 ± 0.036**	0.814 ± 0.052****	0.47 ± 0.064	0.694 ± 0.032**	0.756 ± 0.062***	0.874 ± 0.081****
C18	0.266 ± 0.041	0.296 ± 0.033	0.318 ± 0.045	0.368 ± 0.015	0.268 ± 0.019	0.26 ± 0.018	0.296 ± 0.035	0.314 ± 0.014
C18:2-OH	0.026 ± 0.004	0.038 ± 0.01	0.036 ± 0.002	0.038 ± 0.012	0.022 ± 0.004	0.036 ± 0.007	0.03 ± 0.003	0.032 ± 0.006
C18:1-OH	0.046 ± 0.014	0.064 ± 0.013	0.052 ± 0.007	0.062 ± 0.012	0.036 ± 0.002	0.062 ± 0.008	0.076 ± 0.014*	0.066 ± 0.006
C18-OH	0.028 ± 0.004	0.032 ± 0.007	0.04 ± 0.005	0.034 ± 0.005	0.024 ± 0.007	0.024 ± 0.002	0.018 ± 0.004 #	0.034 ± 0.006

Symbol	Significance
*	Effect of time; *p<0.05; **p<0.01; ***p<0.005; ****p<0.001
#	Lox/Lox vs. Cpt2A-/-; #p<0.05;

**Table S2. Gene abbreviations and primer sequences, related to Experimental Procedures.**

Abbreviation	Gene Symbol	Gene name	Forward primer	Reverse primer
Cpt2	Cpt2	Carnitine palmitoyltransferase 2	CAACTCGTATACCCAAACCCAGTC	GTTCCCATCTGATCGAGGACATC
Cpt1b	Cpt1b	Carnitine palmitoyltransferase 1b, muscle	GGTCCCATAAGAAACAAGACCTCC	CAGAAAGTACCTCAGGCCAGGAAAG
Mcad	Acadm	Acyl-Coenzyme A dehydrogenase, medium chain	AACACTTACTATGCCTCGATTGCA	CCATAGCTCGAAAATCTGAA
Ucp-1	Ucp1	Uncoupling protein 1 (mitochondrial, proton carrier)	GTGAAGGTCAAGCAAGC	AGGGCCCCCTTCATGAGGTC
Pgc1a	Ppargc1a	Peroxisome proliferator activated receptor, gamma, coactivator 1 alpha	CAACATGCTCAAGCCAAACCAACA	CGCTCAATAGTCTTGTCTCAAATGGG
Elovl6	Elovl6	Elovl family member 6, elongation of long chain fatty acids	TCAGCAAAGCACCAGAAC	AGCGACCATGTCTTGTAGGAG
Dio2	Dio2	Deiodinase, iodothyronine, type II	AATTATGCCCTGGAGAAAGCCG	GGCAGTTGCCTAGTGAAAGGT
Prdm16	Prdm16	PR Domain containing 16	CAGCACGGTGAAGCATT	GGGTGCATCCCTGTG
Ntrk3	Ntrk3	neurotrophic tyrosine kinase, receptor, type 3	TGGCTCACACTGATCTCTGG	GCCAGAGCCTTACTGCATC
Otop1	Otop1	Otopetrin 1	ACTAGGACCCCGTCGAATCT	ACCATGCTCTACGTGCTGTG
Irf4	Irf4	Interferon regulatory factor 4	CAGGACTACAATCGTAGGGAGG	GCACATCGTAATCTGTCTTCA
Ppara	Ppara	Peroxisome proliferator activated receptor alpha	GGCTACGGCAATGGTTTAT	GAACGGCTTCTCAGGGTCTT
Acot1	Acot1	Acyl-Coenzyme A thioesterase 1	GACAAGAAGAGCTTCACTCCGTG	CATCAGCATAGAACTCGCTCTCC
Acot2	Acot2	Acyl-Coenzyme A thioesterase 2	AGTCAACGACGCAAAATGGT	GCTCTTCAAATCTGTGGC
Acot4	Acot4	Acyl-Coenzyme A thioesterase 4	ACATCCAAGGTAAGGGCCA	TCCACTGAATGCAAGGCCATT
Acot5	Acot5	Acyl-Coenzyme A thioesterase 5	ACCTCAGGTCAAGGGTCA	TGAAATAACGTTGACCGGGC
Ehhadh	Ehhadh	Enoyl-coenzyme A, hydratase/3-hydroxyacyl coenzyme A dehydrogenase	CAGATGAAGCACTCAAGCTT	ACCTGGCAATGGCTCTGCA
Pgc1B	Ppargc1b	Peroxisome proliferator activated receptor, gamma, coactivator 1 beta	GCCTCTCAGGCAAGTTCA	TAGAGAACTCAGTCCAGAAGGCTT
Fabp3	Fabp3	Fatty acid binding protein 3, muscle and heart	ACCTGGAAGCTAGTGACAG	TGATGTTAGTAGGGCTGTGAT
Gyk	Gyk	Glycerol kinase	CCGCGAAGAAAGCAGTTCTG	CAAAAAACGTGTCGAGCTGGTA
Pdp2	Pdp2	Pyruvate dehydrogenase phosphatase catalytic subunit 2	ACGAGGATACGAGGCTGAAA	CGATTCCTTGAGAATTGAAAG
Pdk4	Pdk4	Pyruvate dehydrogenase kinase, isoenzyme 4	ATCTAACATCGCAGAAATTAAACC	GGACGTACACAATGTTGATTG
Acs11	Acs11	Acyl-Coenzyme A synthetase long-chain family member 1	ATCTGGTGGAAACGGAGCAAG	TCCTTGGGGTTGCTGTAG
Acs13	Acs13	Acyl-Coenzyme A synthetase long-chain family member 3	TGTCTTCTCATGGATGCCGA	CAGCACGGATGTGCTCTT
Gpd2	Gpd2	Glycerol phosphate dehydrogenase 2	CCACGGTGGTGTGCGATAC	AGGGCTTCTTCACATCCCTA
Pank1	Pank1	Pantothenate kinase 1	ATGACTTGCCTCATTTGAT	TGGGAGCCCCTCAAATT
Slc25a42	Slc25a42	Solute carrier family 25, member 42	CCCTGGACGGACCAAGAT	TGTATTCTCGTGTGCGCTGA
Pyrin1	Pyrin1	Pyrin and HIN domain family, member 1	AAATGCAAGATCAGTCAGCAGAG	TGTCCTGTCCTTTAGCTTT
Ifit1	Ifit1	Interferon-induced protein with tetratricopeptide repeats 1	CAAGGCAGGTTCTGAGGAG	GACCTGGTCACCATCAGCAT
Ifit3	Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	TTCCCGCAGCACGAAACAA	AAATTCAGGTGAAATGGCA
Usp18	Usp18	Ubiquitin specific peptidase 18	GAGAGGACCATAGAAGAGGA	TAACCAACACGACCATGAG
Isg15	Isg15	ISG15 ubiquitin-like modifier	CTAGAGCTAGAGCCTGCG	AGTTAGTCACGGACACCG
Irf7	Irf7	Interferon regulatory factor 7	CAATTCAAGGGATCCAGTTG	AGCATTGCTGAGGCTCACTT
Viperin	Rsd2	Radical S-adenosyl methionine domain containing 2	ATAGTGAGCAATGGCAGCT	AACCTGCTCATCGAACGCTGT
Ifi44	Ifi44	Interferon-induced protein 44	CTGATTACAAAAGAAGACATGACAGAC	AGGCAAAACCAAAGACTCCA
Ckmt1	Ckmt1	Creatine kinase, mitochondrial 1, ubiquitous	TGAGGGAGACCTATGAGGTATTG	TCATCAAAGTAGCCAGAACCGA
Ckmt2	Ckmt2	Creatine kinase, mitochondrial 2	GCATGGTGGCTGTGATGAG	AAACTGCCCCTGAGTAATCTTG
Slc6a8	Slc6a8	Solute carrier family 6 (neurotransmitter transporter, creatine), member 8	TGCAATATCTCAAAGGTGGCAG	CTACAAACTGGCTTCCAGA
Phospho1	Phospho1	Phosphatase, orphan 1	AAGCACATCATCACAGTCCCC	TTGGTCTTCAAGCTGTATCCAG
Cyclophilin A	Ppia	Peptidylprolyl isomerase A	AGCACTGGGAGAAAGGATT	CATGCCCTCTTCACCTTC
18s	Rn18s	18s ribosomal RNA	GCAATTATCCCCATGAACG	GGCCTCACTAAACCATCCAA